# AN AIR-ABRASIVE METHOD FOR THE MICRODISSECTION OF MINERALIZED TISSUES[1]

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Biologically mineralized tissues are often composed of several histologically distinct tissue layers containing inorganic crystals of different structure, size, shape, and orientation, and organic matrices of varying composition and structure. In order to study the molecular structure, organization, and composition of the inorganic crystals and the organic matrices of the individual layers by both biophysical and biochemical methods, it is necessary that the individual histological entities be separated without contamination from adjoining layers, which in many cases interdigitate at their junction. Furthermore, it may also be necessary, in the case of external or exposed surfaces, to remove extraneous deposits or layers of material covering the surface without the use of chemical agents, which may alter the underlying inorganic crystals, or leach out various soluble organic components. In order to obtain such histologically defined material, the samples must be prepared by microdissection. This is a difficult technical task, however, because of the extreme hardness and the firm adherence of the interdigitating layers.

The necessity of obtaining histologically characterized tissue and the problems involved in the preparation of such samples are well illustrated by reference to one of the current projects under investigation in these laboratories, the characterization of the structural proteins of mature, erupted, bovine dental enamel.

Since the enamel of mature bovine teeth contains approximately 0.06% protein by weight (Glimcher, Friberg and Levine, unpublished data) and is situated between a layer of dentin and cementum (Glimcher, Friberg and Levine, 1963), each of which contains approximately 30% protein (primarily collagen) by weight, a minute contamination from either source in the enamel sample would result in a very serious and misleading error in an analysis of the organic matrix of enamel. For example, 70% to 80% of the proteins derived from a sample of enamel containing 1% dentin or cementum would contain 70% to 80% collagen and only 20% or 30% enamel proteins. In order to characterize the enamel proteins, it is

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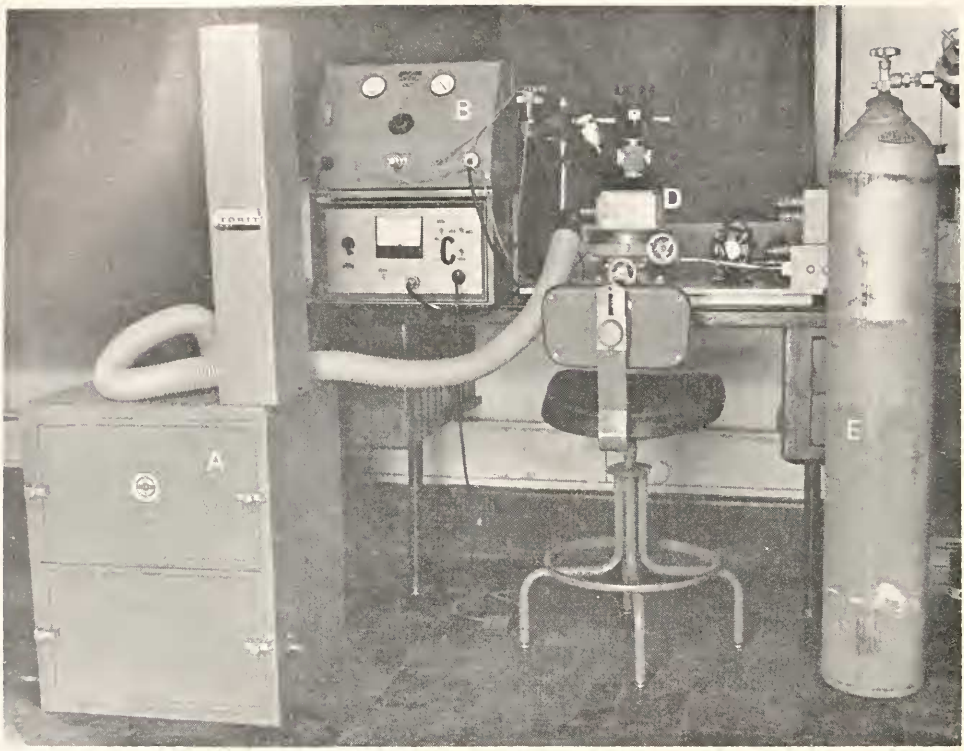


FIGURE 1. Overall view of apparatus: (A) dust collector; (B) air-abrasive unit over (C) master control unit; (D) microdissection unit; (E) gas cylinder.

therefore essential that the samples be free of the adjacent layers of dentin and cementum. Furthermore, because of the very low protein content of the enamel, large amounts of tissue are required for the analyses.

In this report, a method is described for the sectioning of enamel and other mineralized tissues, employing the principle of air-abrasive cutting. Sectioning of the fully mineralized samples is accomplished by feeding compressed air or gas through a vibrating chamber filled with abrasive powder and directing the resultant air-abrasive stream at supersonic speed through a fine nozzle at the object to be cut. This technique is widely used in industry to provide cool, non-contact cutting of materials such as mica, glass, crystals, ceramics, refractory metals, and others that would be likely to shatter with the usual contact methods. In a recent non-industrial application this procedure has been used to uncover rock-embedded fossil material (Stucker, 1961).

In order to employ the air-abrasive method of cutting for the microdissection of mineralized tissues a special apparatus was designed and constructed. This apparatus allows mineralized tissue sections to be mounted on a motorized cross-feed stage and passed under direct microscopic viewing below a fixed nozzle delivering the air-abrasive jet.

## Experimental

*Description of the apparatus*

An overall view of the apparatus is presented in Figure 1. It consists of the following 5 subunits: air-abrasive unit, master control unit, microdissection unit, dust collector, and gas source.

*The air-abrasive unit* shown in Figure 2 (Model C Industrial Air-abrasive Unit, S. S. White) is used without internal modification. It is placed on a shelf over the master control unit (Fig. 2) to which all external electrical connections are made.

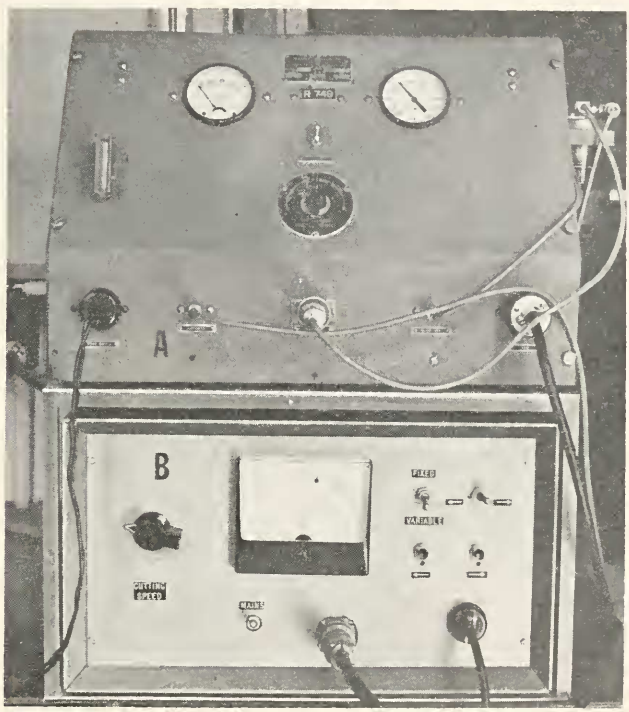


Figure 2. Air-abrasive unit (A) mounted above master control unit (B). Individual controls are labeled.

The master control unit consists of various controls and two D. C. supply units; the air-abrasive unit and the dust collector are connected to the mains through it. One of the D. C. supply units delivers a fixed voltage of 120 V to relay coils and electromagnetic clutches as well as to the field winding of the table-drive shunt motor. The armature winding is connected to the second D. C. supply, which is adjusted from 0 to 120 V through the use of a variable auto-transformer. The output speed of the motor is proportional to the armature voltage, which is indicated by a volt-meter. Positioning of the specimen table at full armature voltage is effected through two, mutually exclusive push-buttons. Table direction during the cutting is preset with a toggle switch and the speed is set by means of the

variable transformer. During microdissection, the motorized table-drive is controlled through a foot-switch, which simultaneously activates the air-abrasive stream and the dust collector.

*The microdissection unit* consists of a specimen stage and a stereo microscope mounted on a steel base together with the drive motor and a two-speed gearbox (Fig. 3). A cross-feed rotary milling table, which is horizontally mounted, serves as the specimen stage. The section holder is placed on top of this unit. The section holder is made of a one-half inch brass block with a spring clamp in a dust enclosure box (Fig. 4). Horizontal motion of the table (from left to right, or right to left) is effected by means of the shunt motor. The crank wheels for the cross-feed and table rotation are controlled manually. One full turn of the crank-wheel corresponds to 0.1″ travel or 9° rotation, respectively.

*The dust enclosure box* (Figs. 3, 4) is made of acrylic resin except for a glass top, which can be replaced when vision becomes impaired due to the action of the

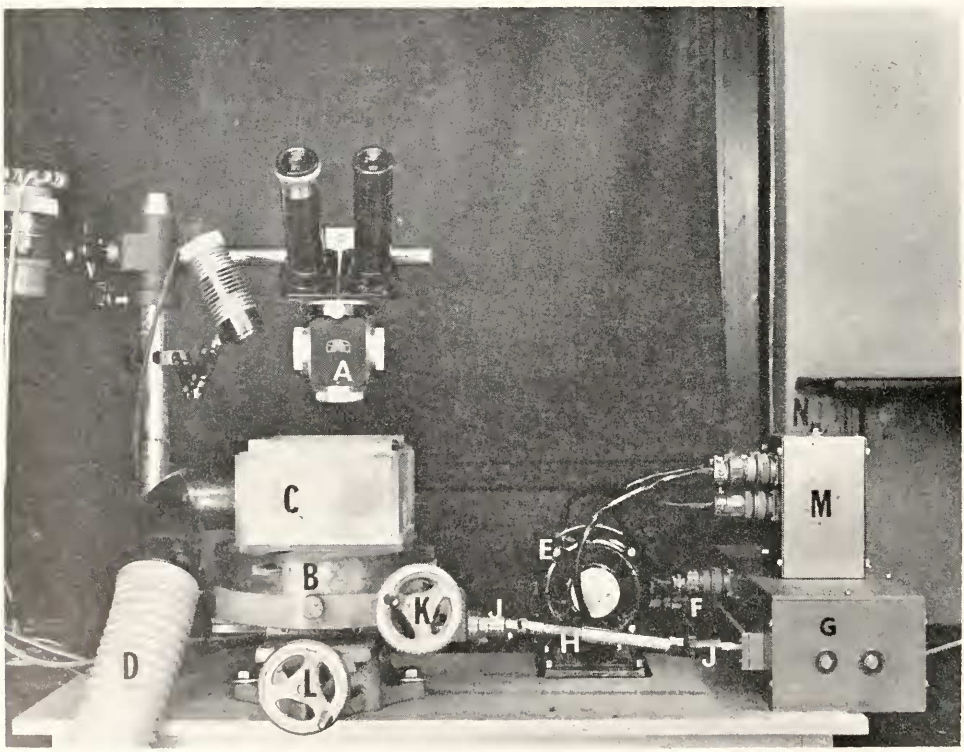


FIGURE 3. Microdissection unit: The stereo microscope (A) is mounted above the cross-feed stage (B). The dust enclosure box (C) contains the specimen holder and is connected to the collector hose (D). The drive motor (E) is connected through a flexible shaft coupling (F) to the input shaft of the two speed gearbox (G). The output of the gearbox is connected to the specimen table by an extension shaft (H) and universal joints (J). The wheel cranks (K) and (L) manually control rotary and fore-and-aft motion of the specimen table during dissection. The coupling box (M) is used to disconnect the motor temporarily when making right-angle end cuts by means of the push button (N).

abrasive. The floor of the dust enclosure is protected by a piece of hard rubber. The hand piece, which extends into the dust enclosure box through a rectangular opening in the rear wall, is mounted so that the nozzle tip is tilted a few degrees from a vertical position. This permits the operator to observe the end of the nozzle tip and its position in relation to the specimen through the stereo microscope during the actual dissection. A metal wire mesh over the vacuum suction opening prevents loss of dissected specimens. In order to prevent the air-abrasive stream from cutting the section holder, the tissue sections are mounted so that they extend

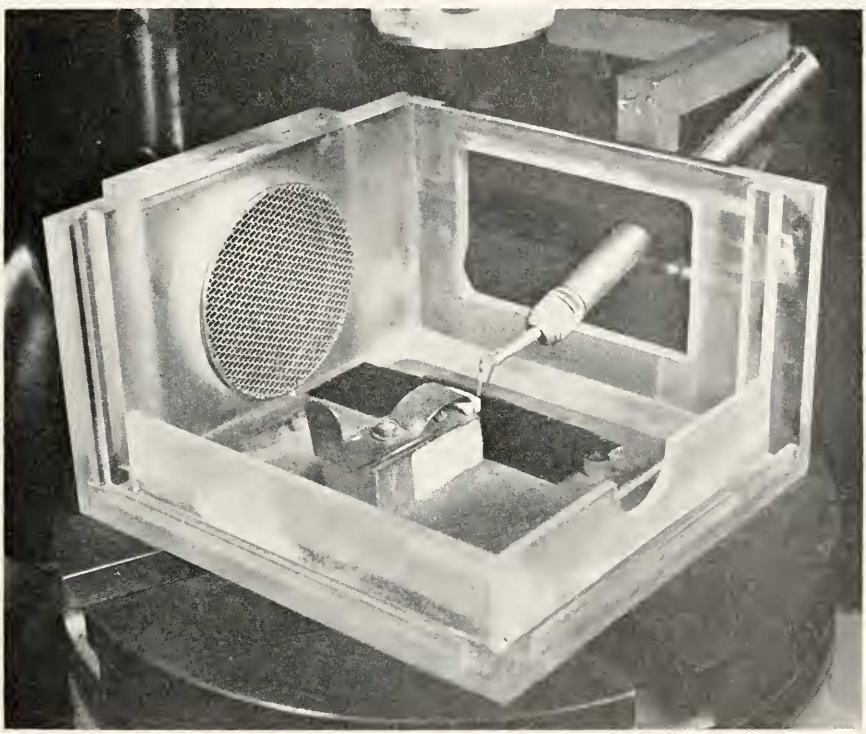

FIGURE 4. Cross-section of tooth crown mounted in dust enclosure box with nozzle positioned for cutting.

$\frac{1}{2}$ inch over the edge of the brass block. Illumination of the sections during microdissection is provided by an overhead lamp, which is mounted (as is the stereo microscope) on a vertical steel rod.

*The handpiece* (Fig. 4), with a right-angle nozzle tip, is mounted in a fixture and may be raised and lowered through a rack and pinion drive (Fig. 4). A variety of interchangeable nozzle tips are available with rectangular and circular orifices and with tip angles of 90°, 135°, and 180°. The rectangular orifice nozzles are available as 0.003″ × 0.060″ or 0.006″ × 0.060″. Circular orifice nozzles are made with 0.010″, 0.018″, and 0.026″ diameter. A rectangular orifice nozzle is used when narrow, rectilinear cuts are required. The minimum width of the cutting

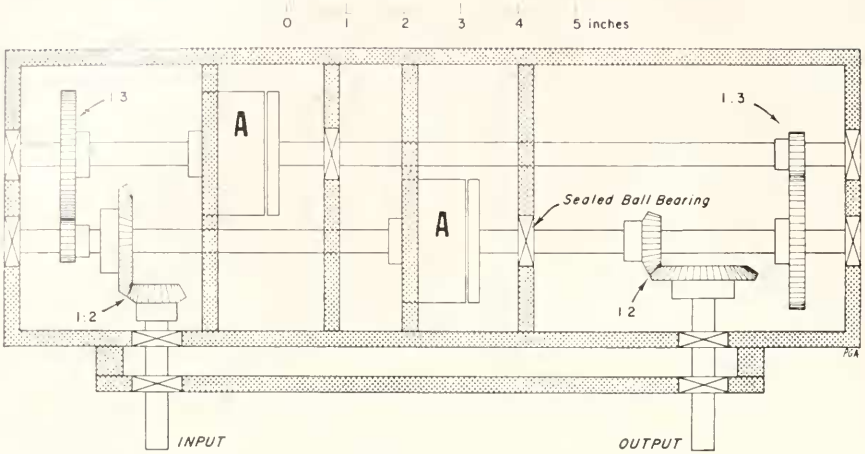


FIGURE 5. Two speed gearbox with electromagnetic clutches (A) for remote control.

path is approximately 100 to 200 microns for the rectangular orifice nozzles, and 300, 500, and 700 microns for the circular orifices. For curvilinear cutting, as in the preparation of enamel, the use of nozzles with circular orifices is preferable.

The drive motor is a 1/15 hp D. C. shunt motor with a 1:10 worm gear and a nominal output speed of 173 rpm (Bodine NSH-34R11). It is connected through a flexible shaft coupling to the input shaft of the two-speed gearbox. The latter is built from $\frac{1}{4}$" sheet brass and has 24 pitch bronze gears and $\frac{3}{8}$" shafts running in sealed ball bearings (Fig. 5). The gear ratios of 1:4 and 1:36 are remotely selected through two electromagnetic clutches (Sterans 2.5 SMR). The 1:4 ratio

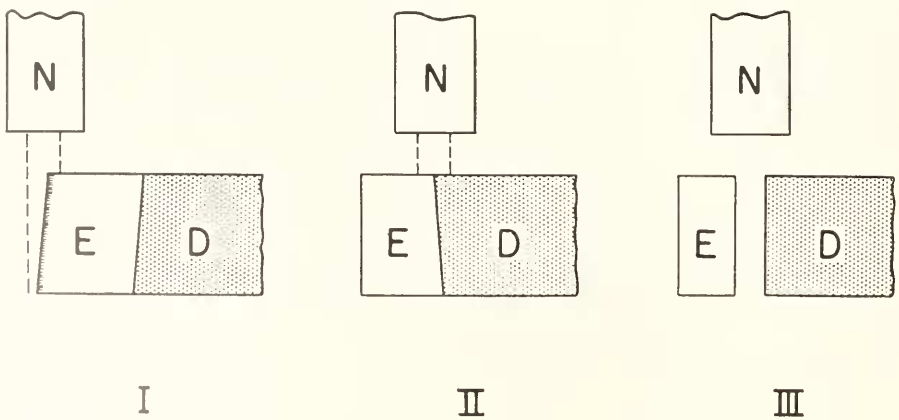


FIGURE 6. Diagramatic representation of the position of the specimen in relation to the nozzle (N) during dissection. I. Removal of cementum (horizontal shaded surface) and outer one-fourth of the enamel (E). II. Position of the nozzle at the junction of dentin (D) and enamel (E) after specimen has been turned over. Note that the inner one-fourth of the enamel is also sacrificed. III. Slab of enamel fully dissected.

is used with full motor speed for pushbutton positioning of the table. The 1:36 ratio is used for cutting in conjunction with a variable motor speed. Under prevailing load conditions, the gearbox output is 50 rpm at positioning, and 0.7 to 5.5 rpm at cutting, corresponding to table speeds of 5 ipm and 0.07 to 0.55 ipm, respectively. The output shaft of the gearbox is connected to the specimen table through universal joints and an extension shaft. Electrical connections from the master control unit to motor and gearbox run through a coupling box and are used to disconnect the motor temporarily when making right-angle end cuts.

To prevent the abrasive powder from being disseminated throughout the work area, the dust enclosure is connected through a flexible hose to an industrial dust collector (Torit Model 54). Noise and vibration have been minimized by fitting the dust collector with an exhaust silencer and shockmounts.

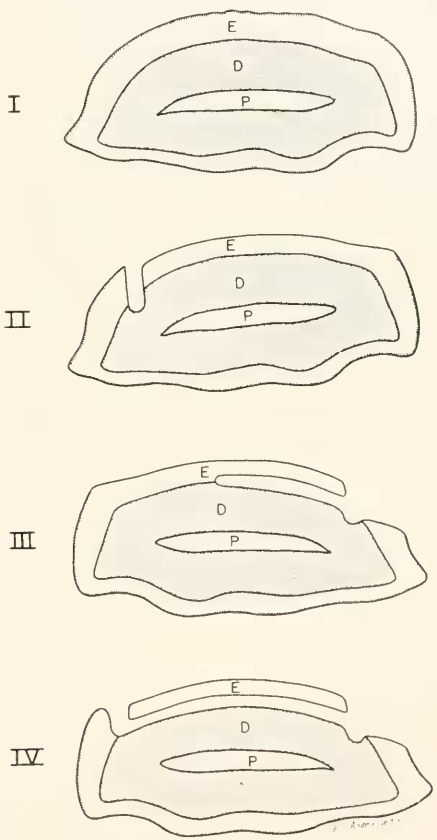


FIGURE 7. Schematic diagram of the dissection of the enamel as viewed through a stereo microscope. I. Appearance of the 1600-micron-thick cross-section of the crown of a bovine incisor tooth. Outer layer of cementum is shown by the short black lines at the surface. E = enamel; D = dentin; P = pulp space. II. The layer of cementum and the outer one-fourth of the enamel have been removed. III. Section turned over and dissection started at the dentino-enamel junction. IV. Slab of enamel completely dissected.

The gas source is a cylinder of nitrogen gas. Carbon dioxide, or a line supply of compressed air, if available at the required pressure, 80–100 psi, may also be used as the propellant gas.

*Methods*

To illustrate the use of the apparatus, the microdissection of the fully calcified enamel of erupted mature bovine incisor teeth will be described in some detail. Serial cross-sections of the crowns of 18- to 36-month-old steers are cut at about 1600 microns, using a modification of the Gillings-Hamco thin sectioning machine (Friberg and Levine, unpublished data). Because of the curvature of the tooth

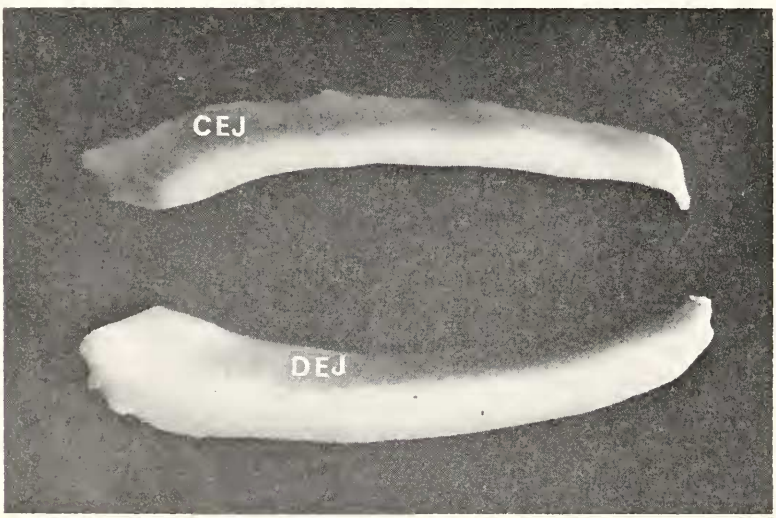


FIGURE 8. Slabs of enamel dissected by air-abrasive technique. DEJ = Enamel surface cut proximal to the dentino-enamel junction; CEJ = Enamel surface cut distal to the cemento-enamel junction. × 8.5.

crown, it is not possible to obtain sections which are consistently at right angles to the surface. To compensate for this, the cross-section is first placed with the outer layer of cementum which covers the enamel sloping downward and outward (Fig. 6, I). The outer one-third to one-fourth of the enamel is then removed by the air-abrasive stream by cutting longitudinally from right to left, terminating with a right angle cut down into the dentin (Fig. 7, II). The section is next turned over so that the slope of the dentino-enamel junction is downward-inward (Fig. 6, II). A second longitudinal cut is made along the dentino-enamel junction, again from right to left, sacrificing approximately the inner one-fourth to one-third of the enamel and a corresponding width of the dentin (Figs. 6 II, 7 III). Finally, a right-angle end cut is made up through the enamel, isolating a slab of enamel, corresponding roughly to the middle one-half to one-third of the original enamel layer (Figs. 6 III, 7 IV, 8). Relatively large samples (50 gm.) of enamel were readily

prepared by this method from the labial side of the coronal surface only, since the enamel covering the lingual surface was too thin.

A second application of this technique has been the microdissection of the prismatic and nacreous layers of the shell of the mollusc, *Mercenaria mercenaria*. In this instance, however, because of the size and shape of the shell, it was difficult to cut the 1600-micron cross-sections of the shell in the Gillings-Hamco apparatus.

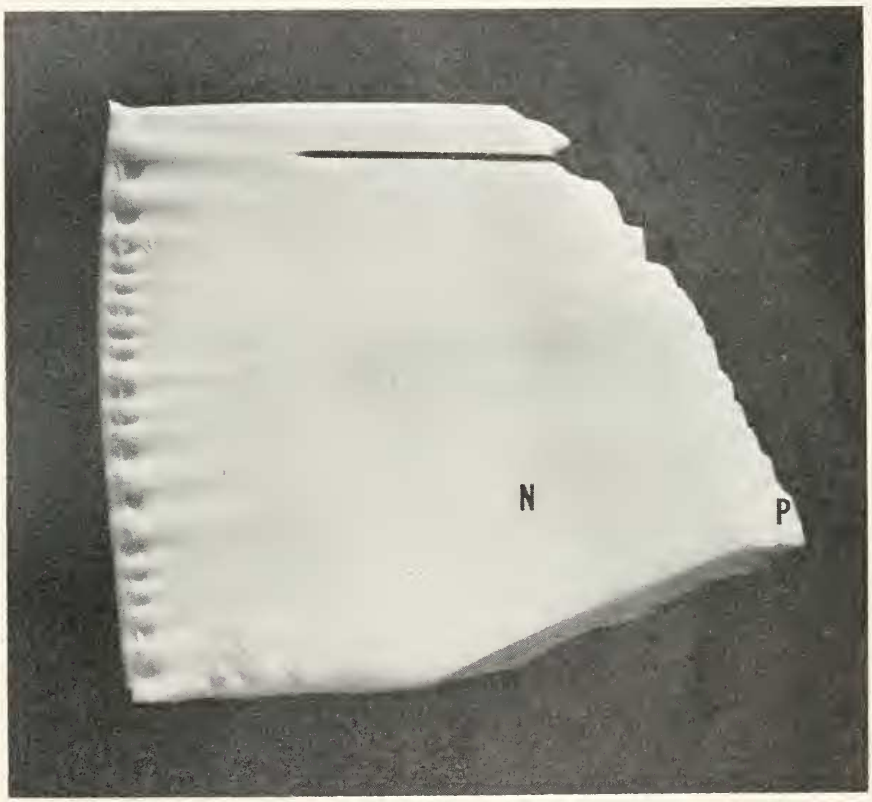


FIGURE 9. Lip portion of shell of *Mercenaria mercenaria* from which a slab has been partially cut, using a rectangular nozzle (0.006″ × 0.060″). This is to be used for the dissection of the prismatic layer. × 8.5. N = nacreous layer; P = prismatic layer.

These were readily prepared, however, by air-abrasive dissection using the 0.006″ × 0.060″ rectangular nozzle (Fig. 9). In a fashion similar to that described for enamel, the periostracum and the outer one-fourth of the prismatic layer were first removed from cross-sections of the shell, following which a slab of pure prismatic layer was dissected by air-abrasive cutting along the junction of the prismatic and nacreous layers (Fig. 10). The nacreous layer can be similarly isolated.

The microdissection technique has also been used in the preparation of oriented blocks of mineralized material for electron microscopy and x-ray diffraction studies. For example, 300- to 500-micron-thick coronal or cross-sections of osmium-fixed,

methacrylate- or epoxy resin-embedded teeth, were cut into strips 500 microns wide by air-abrasive microdissection. From such strips, segments of appropriate length from known locations and orientations can easily be cut. The segments are then re-embedded and sectioned on an ultramicrotome for use in electron microscopy. For x-ray diffraction studies, strips, slabs, rods, cubes, etc. of known location and orientation may be dissected directly from the tissue. Since the optimum thickness of mineralized tissues for x-ray diffraction is often less than 100 microns, further dissection by the air-abrasive method or hand grinding may be necessary.

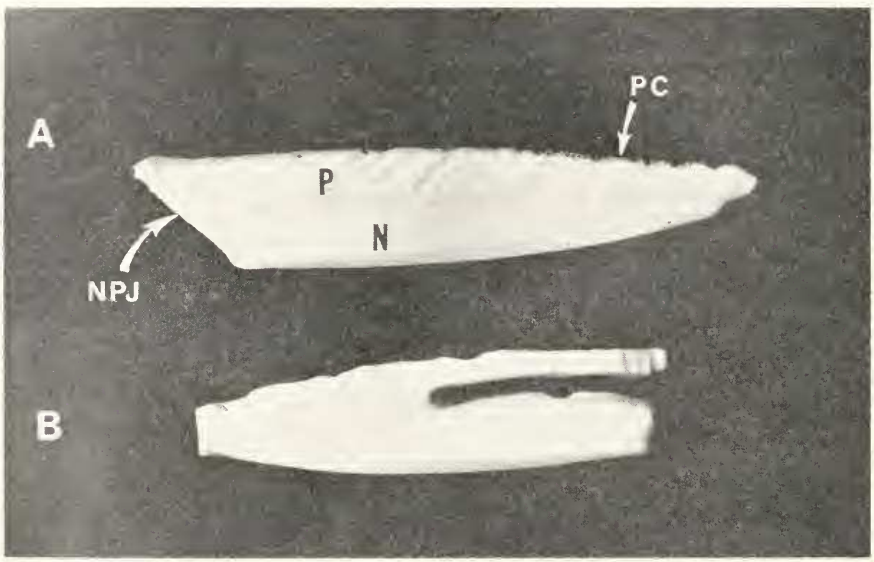


FIGURE 10. Preparation of a slab of prismatic layer from section prepared as in Figure 9. (A) original section; (B) after removal of the periostracum, a slab of the prismatic layer is being dissected by sectioning along the junction between the prismatic and nacreous layers. × 8.5. P = prismatic layer; N = nacreous layer; PC = periostracum and surface contaminants; NPJ = nacreous-prismatic junction.

## DISCUSSION

Histologically defined tissue layers have been prepared from fully mineralized samples of adult bovine teeth and other highly mineralized structures, such as molluscan shells, by microdissection without prior chemical modification, utilizing a method which employs high speed, gas-impelled, abrasive particles to section the tissue. In the case of the molluscan shells, air-abrasive sectioning can also be utilized for the preliminary preparation of the cross-sections of the shells from which the individual layers are to be dissected. The air-abrasive method for the initial preparation is actually preferable to the conventional method utilizing a rotating disc (Gillings-Hamco or similar devices), in that it eliminates heating, wetting, and shattering of the sample.

Since the air-abrasive stream spreads from the orifice in conical fashion it is usually best not to exceed a section thickness of 2 mm. in order to maintain pre-

cision in microdissection; 1.6 mm. was found to be optimal for the enamel work. However, when the apparatus is used to obtain the initial cross-sections, a thickness of 3 mm. to 4 mm. can be tolerated. In the microdissection of blocks for electron microscopy or x-ray diffraction, sections of 500 microns or less can be handled if supported by a glass slide during cutting. For thin sections it is also advisable to reduce air pressure and abrasive feed and to increase cutting speed. The use of abrasive particles of much smaller size in combination with smaller orifice nozzles is under investigation for use in the preparation of smaller and thinner samples for micro-x-ray diffraction.

For precise work, it is essential that the specimen stage be motor-driven to provide slow, steady motion in the cutting direction during microdissection. The operator is thereby able to concentrate on tracing the respective tissue layer margins. In its present version, the apparatus described provides motor drive for the stage in one direction only, *viz.*, right to left, or reverse. Manual control of the cross-feed was retained since it was found to be adequate for the cutting of rectangular or slightly curved strips. In the preparation of bovine enamel, the surface cemental layer and the dentino-enamel junction of a section can readily be followed by small movements of the cross-feed crank wheel. The rotary feed is not used in the preparation of the enamel strips, but is of value in tracing more strongly curved lines. It would be advantageous for the routine preparation of circular or markedly curved structures also to motorize the cross-feed of the table, and to adopt a "joy-stick" control of the two motors, so that constant vectorial speed can be maintained during the microdissection.

The authors are indebted to Mr. O. Fontain for help with the mechanical construction of the apparatus, and to Mr. R. Cavicchi for assistance with the electrical circuitry.

## Summary

A method for the microdissection of mineralized tissues is described, utilizing high speed, gas-impelled abrasive for the sectioning. The method was developed to make possible the preparation of histologically defined samples of such tissues for biochemical and biophysical analysis. Mineralized tissue sections are mounted on a motorized, cross-feed stage and passed below a fixed nozzle delivering the abrasive stream. The operator controls the direction and progress of the microdissection through a stereo microscope. This procedure may be readily adapted to a wide variety of preparatory tasks involved in the study of mineralized tissues.

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